ELSEVIER

European Neuropsychopharmacology 14 (2004) 175–183

EUROPEAN NEURO-PSYCHOPHARMACOLOGY

www.elsevier.com/locate/euroneuro

Review

Glutamate and anxiety

Veerle Bergink*, Harold J.G.M van Megen and, Herman G.M. Westenberg

Rudolf Magnus Institute of Neuroscience, Department of Psychiatry, B01.206, University Medical Center Utrecht, Heidelberglaan 100, 3584 CX Utrecht, The Netherlands

Received 28 February 2003; received in revised form 24 June 2003; accepted 3 July 2003

Abstract

Although glutamate is a simple molecule, its actions in the limbic system and areas concerning anxiety are complex and widespread. These actions are mediated through different combinations of ionotropic and metabotropic glutamate receptors. Preclinical studies have shown that compounds active at NMDA, AMPA/kaïnate and metabotropic receptors might have anxiolytic properties. The major research effort so far has been directed towards the development of compounds which modulate the function of NMDA receptors. In general, the utility of NMDA and AMPA/kaïnate antagonists is greatly hampered by adverse effects. For the treatment of clinical anxiety disorder a more delicate regulation of the glutaminergic system is required. It is encouraging that different ways to fine-tune the glutaminergic system are emerging, e.g., modulators of the glycine site and compounds acting at the AMPA receptor. Metabotropic glutamate receptor agonists and antagonists are in particular promising in this respect. It can be expected that selective modulators of glutamate activity will be of great clinical significance for the treatment of anxiety disorders.

Keywords: Anxiety; Glutamate; Fear conditioning; Animal models; Therapeutic

1. Introduction

The neurobiological underpinnings of anxiety have recently received much attention. These studies have produced findings that are important to generate hypotheses about the biology of anxiety. Several neurotransmitters have been implicated in the genesis of anxiety with earlier data highlighting the γ-aminobutyric acid (GABA) system, the locus of action of the benzodiazepines. Apart from the GABA system, other neurotransmitters such as serotonin (5-hydroxytryptamine; 5-HT) and noradrenaline (NE) have been implicated in anxiety disorders. Several neuropeptides such as adrenocorticotropic hormone (ACTH), corticotropic releasing hormone (CRH), neuropeptide-Y (NPY) and cholecystokinin (CCK) also seem to play a role in the pathogenesis of anxiety.

Recent developments in the neurobiology of anxiety have highlighted the neurotransmitter glutamate as an important element in anxiety and anxious behaviour. In most synapses the actions of the inhibitory neurotransmitter GABA are opposed by the effect of glutamate, which is the major excitatory neurotransmitter in the central nervous system in mammals. This review will examine pathophysiological and therapeutic hypotheses of glutamate, its receptors and anxiety.

2. Neurobiology of anxiety

From an evolutionary point of view it may be postulated that the anatomic core of fear/anxiety is represented by a set of interrelated limbic structures: the septo-hippocampal system, certain nuclei of the amygdaloid complex and areas of the hypothalamus, as well as the periaqueductal grey matter of the midbrain (Charney et al., 1996). Their basic functions are to evaluate the extent to which situations are threatening for the individual and to select appropriate responses in order to generate adequate patterns of defence. The hippocampus is thought to play a role in processing of context-related information and the expression of anxiety responses to environmental signals. The periaqueductal grey matter, in addition to its role in endogenous pain suppression, is the caudal pole of a longitudinal organised neural system which modulates fear and anxiety. Although many limbic as well as cortical areas are involved in the behavioural expression of anxiety, the amygdaloid complex is

* Corresponding author. Tel.: +31-30-2509-019; fax: +31-30-2129-205.
E-mail address: v.bergink@azu.nl (V. Bergink).

0924-977X/$ - see front matter © 2004 Published by Elsevier B.V./ECNP.
doi:10.1016/S0924-977X(03)00100-7

thought to play a crucial role (for review see Davidson, 2002; Davis, 1997). A large and consistent literature indicates that electrical activation of the amygdala produces a pattern of behavioural changes in animals that closely resembles a response after a fearful stimulus. In contrast, lesions of the amygdala block reactions to fearful stimuli (Maren, 1996). The amygdala and its many efferent projections represent a central fear system involved in the acquisition, consolidation and expression of conditioned fear (Walker and Davis, 2002). Most of our knowledge about the biological underpinnings of anxiety in the amygdala and limbic circuit has been studied using fear conditioning. Relevant background information about fear conditioning has been summarised by LeDoux (1998). In classical fear conditioning a neutral stimulus, which has little behavioural effect by itself, is consistently paired with a strong aversive stimulus. Following a small number of pairings, the neutral stimulus produces effects formerly only produced by the strong aversive stimulus. This change is not seen when the stimulus is presented in an unpaired fashion (Davis et al., 1994; LeDoux, 1994). A cellular analogue to this classical conditioning can also be made. When a weak input to a postsynaptic cell is paired with activation by a second, stronger signal to the same cell, a small number of pairings will be sufficient to increase the synaptic strengths, resulting in enhancement of the synaptic transmission. Details about these synaptic transmissions in the amygdala have provided important information regarding fear conditioning (Rogan et al., 1997).

Glutamate and GABA are abundant in the amygdala and other limbic and cortical structures. In the treatment and neurobiology of anxiety disorders some interest has been focussed on possible abnormalities in GABA neurotransmission and the benzodiazepine receptor (Haefely, 1990; Ninan et al., 1982). However, thus far attention has been focussed mainly on the role of serotonin (Jones and Blackburn, 2002). In the last decades, controlled clinical studies have demonstrated the therapeutic efficacy of drugs selectively affecting 5-HT receptors in different anxiety disorders. These advances have kindled interest in the role of 5-HT in anxiety resulting in a wealth of data on the morphological and functional aspects of the 5-HT neuronal systems. Early models attributed an anxiogenic function to endogenous 5-HT. Paradoxically, empirical data have provided results pointing to an anxiogenic as well as an anxiolytic role of 5-HT. This dual role assumes two independent 5HT systems performing different behavioural functions. Graeff (1993) hypothesised that 5-HT possibly facilitates defensive behaviour by acting on the amygdala while simultaneously inhibiting active defensive patterns organised in the central grey matter and periphery. Viewed in this way, threatening stimuli could activate brain defence mechanisms and 5-HT systems independently. Consequently, 5-HT is not responsible for defence mechanisms per se but only modulates them. This view assumes an indirect influence of serotonin on anxiety. Several other neurotransmitters and neuropeptides play a role in the complex neuroanatomical pathways in anxiety and fear conditioning (for review see Ninan, 1999). Emerging is the importance of corticotropin-releasing factor secreting neurones in the central amygdala nucleus. CRH is hypothesized to facilitate anxiety reactions by activation of this central nucleus (Shepard et al., 2000). Anxiogenic proportions have been ascribed to other neuropeptides as well, for example cholecystokinin.

The noradrenergic and dopaminergic systems are believed to increase arousal in response to threat. The noradrenergic locus coeruleus stimulate the periaqueductal grey via an indirect pathway through the amygdala (Charney et al., 1996).

3. The glutamate neuronal system

Until 1970 it was not recognised that excitatory amino acids such as glutamate might play a physiological role in brain functioning. Since then it has been discovered that the glutamate system can be found in all parts of the human brain (Collingridge and Lester, 1989). This ubiquitous nature of glutamate excitation supports a role for glutaminergic neurotransmission in several cerebral functions (Danysz et al., 1995). For instance, the glutamate system is known to play an important role in cognition, learning and memory (Davis et al., 1994; Maren, 1996; LeDoux, 1994), the neural plasticity of synaptic connections (Kaczmarek et al., 1997), pain perception (Klepstad et al., 1990) and in the regulation of neuroendocrine secretion (Brann and Mahesh, 1994). The spectrum of excitation by glutamate ranges from normal neurotransmission, to excess neurotransmission causing pathological symptoms such as mania or panic, to excitotoxicity resulting in minor damage to dendrites. Hyperactivity of the glutamate system is associated with several neurodegenerative diseases, e.g., Parkinson's disease, Alzheimer's disease, Huntington's disease, amyotrophic lateral sclerosis and schizophrenia (Danysz et al., 1995). The glutamate system might impact progressive neurodegeneration by an excitotoxic mechanism. Slow progressive excitotoxicity can be associated with degeneration such as seen in Alzheimer's disease. Sudden and catastrophic excitotoxicity can cause neurodegeneration as in stroke (Cotman et al., 1995).

3.1. Glutamate receptors

For years the central effects of glutamate were thought to be exclusively mediated by ion channel mechanisms. However, glutamate receptors can now be categorised into two major groups, (I) ionotropic and (II) metabotropic receptors. This categorisation is based on intracellular/extracellular coupling and on different pharmacological and biochemical characteristics. Ionotropic receptors can be subdivided into

N-methyl-D-aspartate (NMDA), kaïnate and quisqualate receptors named after the agonists that selectively bind to these receptors. These synthetic selective agonists resemble either glutamate or aspartate. The quisqualate receptor has been renamed as the amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptor (Hollmann and Heinemann, 1994; Nakanishi, 1992).

The NMDA receptor-channel complex has several characteristic features. There are several regulatory sites on this NMDA receptor complex. Three of these modulatory sites are outside the ion channel (the neurotransmitter glycine site, the polyamine site and the zinc site) and are excitatory in nature. The inhibitory modulator sites are located inside the ion channel. Magnesium ions can block the calcium channel at one of these sites. The other inhibitory site is sometimes called the 'PCP site' because the psychomimetic agent phencyclidine also binds to this site.

Precise modulation is required for normal neuronal functioning, depolarisation of the NMDA receptor results in a slow rising, long lasting current (Cotman et al., 1995).

In most CNS synapses, NMDA receptors coexist with either AMPA or kaïnate receptors. These latter receptors are thought to be involved in amplification of the glutamate signal. The level of concurrent depolarisation depends on AMPA/kaïnate activation and other modulator signals. Both AMPA and kaïnate receptors mediate fast excitatory synaptic transmission (Cotman et al., 1995).

The ionotropic glutamate receptors are distributed throughout the brain. However, different types of receptors exhibit regional and functional variability. Overall the density of NMDA receptors is high in cortical and limbic regions. The distribution of AMPA and kaïnate receptors is similar to that exhibited by the NMDA receptor, consistent with their common action as a functional pair. The cortical and limbic localisation of these receptors accounts for its effects on cognition, perception and mood (Krystal et al., 1999).

In the 1980s it became apparent that glutamate also acts on a class of non-ionotropic receptors or G-protein bound receptors also termed the 'metabotropic' receptor (Pin and Duvoisin, 1995). These glutamate receptor subtypes are often on the same neurones and in almost all cases interact within neural networks. To date eight metabotropic glutamate receptors (mGluRs) have been cloned. These receptors are present at both presynaptic and postsynaptic sites (Fig. 1). The eight mGluRs are subdivided into three groups, each possessing similar pharmacology and second messenger coupling. Metabotropic glutamate receptor subtypes are also differentially distributed within the CNS. Studies in rats have shown that Group II mGluRs are highly localised in the forebrain regions and limbic structures (Ohishi et al., 1993). This expression of mGluRs in different regions provides a way to fine-tune glutaminergic neuronal transmission within specific synapses (Schoepp and Conn, 1993).

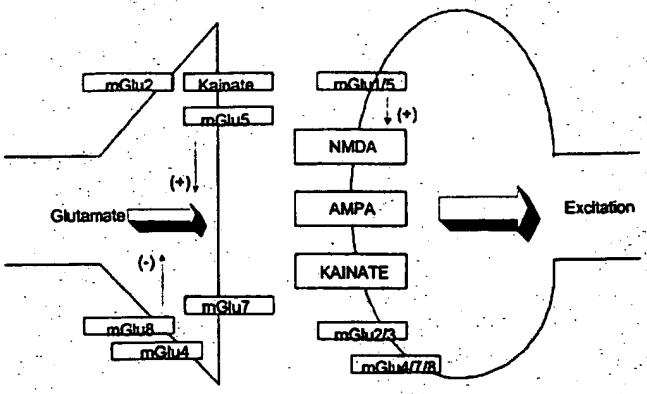

Fig. 1. Glutamate receptor subtypes.

In short, the AMPA and kaïnate receptors evoke fast synaptic responses and in turn promote the activation of voltage-dependent NMDA receptors. The mGluR subtypes exert long-lasting actions through the activation and inhibition of intracellular signals. Coherent cortical function depends on a balanced action of glutamate receptors of different classes.

4. Glutamate and anxiety

The glutaminergic system is thought to play a major role in the pathogenesis of anxiety and fear conditioning.

As mentioned before, treatments that improve the excitability of output neurones in the basolateral amygdala improve aversive conditioning. Alternatively, treatments that decrease the excitability of these neurones produce anxiolytic effects (LeDoux, 1994; Maren, 1996). Decrease in excitatory output in the amygdala can be achieved by decreasing the excitatory glutaminergic transmission. Blocking the basal glutamate excitation generated by ionotropic receptors could elicit a significant anxiolytic effect. Indeed, the administration of antagonists of the NMDA and non-NMDA type receptors into the basolateral amygdala has been shown to reduce anxiety in animal models (Kim and McGaugh, 1992; Miserandino et al., 1990). An alternative way to decrease excitatory output in the amygdala could be achieved by an increase in GABA neurotransmission. The anxiolytic benzodiazepines increase GABA neurotransmission and induce a decrease in excitatory output of the amygdala. There appears to be a balance between GABA receptor mediated inhibition and glutamate receptor mediated excitation that regulates behavioural and physiological responses associated with anxiety (Sajdyk and Shekhar, 1997). In addition, there is mounting evidence, gathered from various parts of the CNS that both inhibitory (GABAergic) and excitatory (glutaminergic) transmission can be modulated by presynaptic excitatory amino acid receptors (Salt and Eaton, 1995). It is likely that these receptors are of the mGluR type(s). Presumably this presynaptic inhi-

bition is achieved through activation of a metabotropic glutaminergic autoreceptor. Thus, GABA receptors produce inhibitory actions in the amygdala. In contrast, glutamate receptors can produce both excitatory and inhibitory actions in the amygdala and the degree of ionotropic and metabotropic activation is likely to be an important determinant of amygdaloid cell excitability (Maren, 1996). These anxiogenic or anxiolytic actions of different glutamate receptors in the amygdaloid cells can be better understood in relation to fear conditioning. Details about the synaptic transmission in the amygdala has been studied using a cellular analogue to fear conditioning (Rogan et al., 1997). In classical fear conditioning, a neutral stimulus elicits release of glutamate onto neurones in the amygdala. This glutamate binds to both NMDA and AMPA/kaïnate receptors. However, this might not produce much of a behavioural response. Only weak activation of AMPA/kaïnate receptors occurs and in addition, the NMDA-channel is not permeable by a partial blockade of Mg^{2+}. However, presentation of a strong aversive stimulus at about the same time can further depolarise the neurone and relieve the Mg^{2+} blockade leading to a behavioural response. This triggers events that increase the ability of the previously neutral stimulus to activate the neurone, enabling it to produce effects similar to those previously produced only by the aversive stimulus (Davis et al., 1994).

NMDA antagonists can prevent this process (Bliss and Collingridge, 1993). The role of NMDA antagonist in fear conditioning based on intra-amygdala injection of NMDA antagonists had been studied by Miserandino et al. (1990) who found that intra-amygdala blockade of NMDA receptor function disrupts acquisition of fear conditioning. However, the application of NMDA alone is usually not sufficient to induce a change. Other stimuli, in addition to NMDA receptor activation, may be required to facilitate the process of fear conditioning. The role of AMPA receptors and metabotropic glutamate receptors was also analysed in this respect (Walker and Davis, 2002). For extensive information on the role of metabotropic receptors in fear conditioning the reader is referred to the article of Watkins and Collingridge (1994).

5. Glutamate in animal models of anxiety

Pharmacological agents, that block glutamate output, may be of therapeutic use for the treatment of anxiety. Glutamate receptor ligands are effective in animal models for anxiety by preventing fear conditioning and by having direct anxiolytic effects. Glutamate agonists and antagonists have been tested in different anxiety paradigms. Generally, two main categories of animal models can be distinguished, conditioned behaviour models and unconditioned behaviour models. Conditioned behaviour models use conflict tests. Examples of unconditioned behaviour models are the social interaction paradigm, the elevated plus maze, the ultra sonic vocalisation paradigm and the acoustic startle paradigm. The glutamate receptor ligands used in several anxiety models are shown in Tables 1–3.

Most studies have focussed on the role of NMDA glutamate receptors (Table 1). There are at least four sites at which antagonists can block the activation of the NMDA complex. Competitive antagonists block the NMDA site itself, non-competitive antagonists like phencyclidine act by blocking the cation channel. Inhibition of NMDA receptor activity could also be achieved via blockade of NMDA/glycine-sensitive sites. Many studies show functional differences between antagonists acting at the different sites associated with the NMDA receptor complex (Table 1), even compounds with similar potencies under in vitro conditions can have different functional profiles in vivo.

Overall, the effects of phencyclidine-like NMDA receptor antagonists are not unequivocal in nature and appear to be less specific as compared to the effects of competitive NMDA receptor antagonists (Wiley, 1997). Consequently, the search for 'NMDA' compounds with anxiolytic properties was focussed on the development of competitive NMDA channel blockers and glycine receptor antagonists (Wiley et al., 1995; Dunn et al., 1989; Bennet and Amrick, 1986).

A limited number of investigations were carried out with non-NMDA receptors ligands (Table 2 and 3) since active and selective ligands for these non-NMDA receptors were only available to a limited extent. AMPA antagonists displayed anxiogenic actions in three studies with conditioned and unconditioned tests (Benvenga et al., 1993; Karcz et al., 1995; Kotlinska and Liljequist, 1998a,b).

Behavioural studies of mGLuR activation or inhibition are also scarce.

The heterogeneous family of metabotropic receptors has only recently been cloned and the discovery of compounds that selectively modulate the receptor is still in its infancy (Schoepp and Conn, 1993). In the last 10 years different groups (i.e., I, II and III) of metabotropic receptors agonists and antagonists have been developed. Although interesting in vitro, most compounds are not yet useful in vivo because of poor bioavailability and low potency. Recently, systemically active mGlu5 receptor antagonists were discovered and one derivative demonstrated anxiolytic potential (Brodkin et al., 2002; Pilc et al., 2002; Spooren et al., 2000; Tatarczynska et al., 2001). A high expression of mGlu5 receptors in the limbic forebrain regions was observed. So far, one metabotropic receptor agonist showed potent central effects when tested systemically (Helton et al., 1997, Klodzinska et al., 1999; Shekhar and Keim, 2000). This compound acts at mGlu2 and mGlu3 receptors, distributed mainly in the limbic system.

Table 1
NMDA receptor antagonists

Substance	Anxiety test	Anxiety	Motor	Authors
Non competitive antagonists				
Ketamine	CT, SI, X-maze	=, ↑		Silvestre et al. (1997)
MK801	CT	↓ =	=, ↑	Xie and Commissaris (1992), Corbett and Dunn (1991), Koek and Colpaert (1991), Jessa et al. (1996)
	SI	↓	=	Corbett and Dunn (1991), Dunn et al. (1989)
	X-maze	↓	=	Corbett and Dunn (1991), Dunn et al. (1989), Fraser et al. (1996)
		=	↑	Criswel et al. (1994)
PCP	Open field		↑	Kotlinska and Liljequist (1998), Plaznik et al. (1994), Jessa et al. (1996)
	USV	↓	↓	Vry De et al. (1993), Kehne et al. (1991)
	CT	↓, =		Porter et al. (1989), Sanger and Jackson (1989)
	X-maze	=		Wiley et al. (1995)
Mem, am	USV	↓		Vry De et al. (1993)
	CT, X-maze	=		Karcz et al. (1997)
Competitive antagonists				
NPC 17742	X-maze, CT	↓	=	Wiley et al. (1995), Willetts et al. (1994)
CPP	CT	↓		Corbett and Dunn (1991), Koek and Colpaert (1991)
CGS 19755	CT	↓		Bennet and Amrick (1986), Koek and Colpaert (1991)
AP5	X-maze, SI, USV	↓		Dunn et al. (1989), Kehne et al. (1991)
AP7	ASP, CT, X-maze, SI			Anthony and Nevins (1993), Bennet and Amrick (1986), Stephens et al. (1986)
				Dunn et al. (1989), Plaznik et al. (1994)
CGP 37849	Open field, CT	↓	=, ↓	Jessa et al. (1996), Plaznik et al. (1994), Przegalinsky et al. (1996)
Glycine site				
ACEA 1021	X-maze	=		Wiley et al. (1995)
HA 966	X-maze, SI, CT, USV			Trullas et al. (1989), Dunn et al. (1992), Anthony and Nevins (1993)
	ASP			Karcz et al. (1997
5,7 DCKA	Open field, CT, USV	↓	↓, =	Plaznik et al. (1994), Kehne et al. (1995), Corbett (1993)
7 CKA	ASP, CT	↓, =		Koek and Colpaert (1991), Anthony and Nevins (1993)
Cycloserine	ASP, X-maze	↓	=	Anthony and Nevins (1993), Karcz et al. (1997)
MDL	USV	↓	=, ↓	Kehne et al. (1995), Baron et al. (1997)
L-701,324	CT, X-maze	↓, =	=	Kotlinska and Liljequist (1998), Karcz et al. (1997)
ACPC	CT, X-maze, ASP	↓	=	Anthony and Nevins (1993), Przegalinsky et al. (1996), Karcz et al. (1997)
Polyamine site				
Ifenprodil	X-maze	↓	↑	Fraser et al. (1996)

Substances: MK 801, dizolcipine; PCP, phencyclidine; CPP, 3-(2-carboxy piperazine-4yl)-propyl-1-phosphonic-acid; CGS 19975, *cis*-4-phosphonomethyl-2-piperidine-carboxylkynurenate; AP5, 2 amino-5-phosphonoheptanoate; AP7, 2 amino-7-phosphonoheptanoate; HA 966, 3-amino-1-hydroxy-2-pyrrolidinone; 5,7 DCKA, 5,7-dichlorokynurenic acid; 7 CKA, 7-chlorokynurenic acid; MDL' 102,288, 5,7-dichloro-1,4-dihydro-(((4-((methoxycarbonyl)amino)-6-chloro-1H-indole-2-carboxylic acid; MDL" 100,458, (3(benzoylmethylamino)-6-chloro1H-indole-2-carboxylic acid; MDL"' 105,519, (*E*)-3-(2-phenyl-2-carboxyethenyl)-4,6-dichloro-1H-indole-2-carboxylic acid; L-701,324, 7-chloro-4-hydroxy-3-(3-phenoxy) phenyl-2(1H)-quinolone; ACPC, 1-aminocyclopropanecarboxylic acid; Memantine, amantine; LY326325; LY215490, 3*SR*, 4*aRS*, 6*RS*, 8*aRS*)-6-(2(1H-tetrazol-5-yl)ethyl)decahydro-isoquinoline-3-carboxylic acid; LY354740, 1*S*, 2*S*, 5*R*, 6*S*-2-aminobicyclo(3.1.0)hexane-2-6-dicarboxylate monohydrate; NBQX, dihydroxy-6-nitro-7-sulfamoyl-benzo(*F*)quinoxaline; MPEP, 2-methyl-6-(phenylethynyl)pyridine.
Anxiety tests: CT, conflict test; X-maze, elevated plus maze; USV, ultrasonic vocalisation; SI, social interaction test; ASP, acoustic startle paradigm.

These preclinical studies indicate that compounds active at NMDA, AMPA/kainate and metabotropic receptors might have anxiolytic properties. However, animal models for anxiety are only predictive to a limited extent and therefore can only be used as a rough screening method for the development of compounds with anxiolytic properties that are clinically effective. Common to conditioned as well as unconditioned tests is their reliance on motor behaviour (Dawson and Tricklebank, 1995). Some 'sedative-like' properties of the glutaminergic ligand could also result in the effects as presented in the tables. Drug induced non-specific enhancement or impairment of performance may confound anxiolytic drug effects. Specific tests on motor performance should be performed to distinguish these

effects from potential anxiogenic or anxiolytic effects. Not all authors have performed such independent tests for locomotor activity.

Furthermore, both conditioned as well as unconditioned animal models only measure direct potential anxiolytic effect. Delayed anxiolytic effects as observed with SSRIs were not found.

6. Clinical prospect of glutamate modulating compounds in anxiety disorder

Since the introduction of phencyclidine (PCP) in the late 1950s (Luby et al., 1959) antagonists of the NMDA glutamate receptor have been important tools for exploring the pathophysiology of neuro-psychiatric disorders. This interest has resulted in several clinical studies describing the effects of NMDA antagonists in normal, healthy subjects. Unfortunately, NMDA antagonists can profoundly affect behaviour and produce serious adverse effects (Willetts et al., 1994; Krystal et al., 1994, Danysz et al., 1995).

Studies in healthy human subjects suggest that NMDA antagonists produce disturbances in identity and perception. Cognitive disturbance and behavioural effects resembling schizophrenia have also been described (Javitt and Zukin, 1991). These behavioural effects could be explained by the dense cortical localisation of NMDA receptors (Krystal et al., 1999). These risks of NMDA-antagonists hampered clinical research. To prevent widespread excitotoxicity, NMDA channel blockers have been used in some clinical trials, e.g., in the treatment of Parkinson's disease or stroke (Brenner et al., 1989; Grotta et al., 1995). Some authors in the field of neuro-degenerative disease suggest that in the future it will be possible to develop NMDA receptor antagonists that are well tolerated (Parsons et al., 1999a,b). For the treatment of anxiety, excessive glutamate exposure in specific areas should be blocked, whereas normal glutaminergic neurotransmission should remain unaffected. Therefore, direct inhibition or excitation of the glutamate system is not a promising approach. By analogy, the GABA-benzodiazepine receptor complex has also several sites at which drugs can produce GABA-mediated effects (Johnston, 1996). Direct stimulation of the GABA-ion channel receptor by barbiturates has resulted in many central side effects. In contrast, benzodiazepines with their indirect mode of action modulate the endogenous GABA release (Haefely, 1990). This leads to a safer therapeutic approach. Analogous to the GABA system a more delicate regulation of the glutamate system would be desirable.

Table 2
AMPA receptor antagonists

Substance	Anxiety test	Anxiety	Motor	Authors
Non-competitive antagonists				
LY326325	CT, X-maze	↓, ↑	=	Karcz et al. (1995), Kotlinska and Liljequist (1998)
LY215490	CT	↓		Benvenga et al. (1993)
NBQX	X-maze	↓, =		Karcz et al. (1995)

Table 3
Metabotropic receptor agonists and antagonist

Substance	Anxiety test	Anxiety	Motor	Authors
Agonists (mGlu 2)				
LY354740	ASP, X-maze	↓	=	Helton et al. (1997)
	CT	↓	↓	Klodzinska et al. (1999)
	SI	↓		Shekhar and Keim (2000)
Antagonists (mGlu 5)				
MPEP	X-maze, CT, SI	↓	=	Spooren et al. (2000)
	X-maze, CT	↓	=	Tatarczynska et al. (2001)
	CT	↓		Pilc et al. (2002)
	USV, ASP	↓	=	Brodkin et al. (2002)

It is encouraging that different ways to fine-tune the glutaminergic system are emerging (Danysz et al., 1995; Parsons et al., 1999a,b). In addition to the NMDA-channel, the glycine site could also be a locus of attention (Dannhardt and Kohl, 1998; Kehne et al., 1995). Compounds acting at the AMPA receptor could also be promising (Karcz et al., 1995; Kotlinska and Liljequist, 1998a,b). An area that seems particularly fruitful for clinical application is the mGluRs system (Schoepp et al., 1999; Pin et al., 1999). mGluR agonists and antagonists have a wide variety of actions on central neurones, which are mediated by both voltage-gated and ligand-gated ion-channels. Activation of the different second messenger systems may have excitatory as well as inhibitory effects. Currently clinical studies evaluating anxiolytic properties of metabotropic agonists are under investigation.

7. Conclusion

In summary, although glutamate is a simple molecule, its action in the limbic system and areas concerning anxiety are complex and widespread (Danysz et al., 1995). These actions are mediated through different combinations of ionotropic and metabotropic glutamate receptors and potentially different sub-unit combinations. Preclinical studies indicated that compounds active at NMDA, AMPA/kainate and metabotropic receptors might have anxiolytic properties (see Tables 1–3). The major research effort so far has been directed towards the development of compounds which modulate the function of NMDA receptors by acting within the NMDA receptor complex. Anxiolytic properties and adverse effects due to muscle relaxant properties differ between the compounds used and the status of the activated sub-unit of the NMDA receptor. However, in general, the utility of NMDA and AMPA/kainate antagonists appeared to be greatly hampered by adverse effects because of interference with receptors throughout the whole CNS and body (Willetts et al., 1994; Krystal et al., 1994, Danysz et al., 1995). This has led to the conclusion that NMDA

receptor antagonism is not a valid therapeutic approach for the treatment of anxiety disorders. From the standpoint of novel therapeutic approaches to the treatment of anxiety a delicate regulation of the glutaminergic system is required. Normal glutaminergic neurotransmission, which takes place in virtually every synapse in the CNS, should be unaffected whereas the effects of excessive glutamate in specific areas should be blocked (Davidson, 2002; Maren, 1996; Parsons et al., 1999a,b).

It is encouraging that different ways to fine-tune the glutaminergic system are emerging. Modulators of the glycine site could be clinically useful, as well as compounds acting at the AMPA receptor (Dannhardt and Kohl, 1998a,b; Karcz et al., 1995; Kehne et al., 1995; Kotlinska and Liljequist, 1998a,b). Particularly promising are metabotropic glutamate agonists and antagonists. They represent a new and novel class of compounds with potential therapeutic efficacy in anxiety without some of the side effects associated with NMDA antagonists. The mGluR pharmacology is expanding rapidly. It is now apparent that several pre- and postsynaptic mechanisms exist by which in situ expressed mGluRs could modulate cell function in the CNS (Schoepp et al., 1999; Pin et al., 1999). To further explore mGluR function, the discovery of potent subtypes of selective mGluR compounds is needed. According to the authors, in future most therapeutic opportunities in anxiety disorders might arise from selective modulation of these metabotropic glutamate receptors. Furthermore it is beyond doubt that the actions of glutamate should not be considered in isolation at individual receptors, as glutamate acts at multiple receptors and is subjected to modulation from several sources. It is to be expected that modulators of glutamate activity may ultimately be of great clinical significance in the treatment of anxiety disorders and in psychiatry in general.

References

Anthony, E.W., Nevins, M.E., 1993. Anxiolytic-like effects of *N*-methyl-D-aspartate-associated glycine receptor ligands in the rat potentiated startle test. Eur. J. Pharmacol. 250, 317–324.

Baron, B.M., Harrison, B.L., Kehne, J.H. et al., 1997. Pharmacologic characterization of MDL 105,519, an NMDA receptor glycine site antagonist. Eur. J. Pharmacol. 323, 181–192.

Bennet, D.A., Amrick, C.L., 1986. 2 amino-7-phosphonoheptanoatic acid produces discriminative stimuli and anticonflict effects similar to diazepam. Life Sci. 39, 2455–2461.

Benvenga, M.J., Leander, J.D., Ornstein, P.L., 1993. Anxiolytic effect of the competitive AMPA antagonist 215490 in a pigeon punished responding model. Soc. Neurosci. 19, 293.

Bliss, T.V.P., Collingridge, G.L., 1993. A synaptic model of memory: long term potentiation in the hippocampus. Nature 361, 31–39.

Brann, D.W., Mahesh, V.B., 1994. Excitatory amino acids, function and significance in reproduction and neuroendocrine regulation. Front. Neuroendocrinol. 15, 3–49.

Brenner, M., Haass, A., Jacobi, P. et al., 1989. Amantine sulphate in treating Parkinson's disease: clinical effects, psychometric tests and serum concentration. J. Neurol. 236, 153–156.

Brodkin, J., Busse, C., Sukoff, S.J. et al., 2002. Anxiolytic-like activity of the mGluR5 antagonist MPEP a comparison with diazepam and buspirone. Pharmacol. Biochem. Behav. 73 (2), 359–366.

Charney, D.S., Nagy, L.M., Brenner, D. et al., 1996. Neurobiological mechanisms of human anxiety. In: Fogel, B.S., Schiffer, R.B., Rao, S.M. Neuropsychiatry. Williams and Wilkins, Baltimore, MD.

Collingridge, G.L., Lester, R.A., 1989. Excitatory amine acid receptors in the vertebrate central nervous system. Pharmacol. Rev. 41, 143–210.

Corbett, R., Dunn, R.W., 1991. Effects of HA-966 on conflict, social interaction and plus maze behaviour. Drug Dev. Res. 24, 201.

Corbett, R., 1993. Effects of 5,7 dichlorokynurenic acid on conflict, social interaction and plus maze behaviour. Neuropharmacology 32 (5), 461–466.

Cotman, C.W., Kahle, J.S., Miler, S.E. et al., 1995. Excitatory amino acid neurotransmission. In: Bloom, F.E., Kupfer, D.J. Psychopharmacology: the Fourth Generation of Progress. Raven Press, New York.

Criswel, H.E., Knapp, D.J., Overstreet, D.H., Breese, G.R., 1994. Effects of ethanol, chordiazepoxide and MK-801 on performance in the elevated plus maze and on locomotor activity. Alcohol. Clin. Exp. Res. 18, 596.

Dannhardt, G., Kohl, B.K., 1998. The glycine site on the NMDA receptor: structure–activity relationships and possible therapeutic applications. Curr. Med. Chem. 5 (4), 253–256.

Danysz, W., Parsons, C.G., Bresink, I., Quack, G., 1995. Glutamate in CNS disorders. Drug News Perspect. 8, 261–277.

Davidson, R.J., 2002. Anxiety and affective style: role of prefrontal cortex and amygdala. Biol. Psychiatry 51 (1), 68–80.

Davis, M., Rainnie, D., Cassel, M., 1994. Neurotransmission in the rat amygdala related to fear and anxiety. Trends Neurosci. 17, 208–214.

Davis, M., 1997. Neurobiology of fear responses: the role of the amygdala. J. Neuropsychiatry Clin. Neurosci. 9, 382–402.

Dawson, G.R., Tricklebank, M.D., 1995. Use of the elevated plus maze in the search for novel anxiolytic agents. Trends Pharmacol. Sci. 16, 33–36.

Dunn, R.W., Corbett, R., Fielding, S., 1989. Effects of 5-HT1a receptor agonists and NMDA receptor antagonists in the social interaction test and the elevated plus maze. Eur. J. Pharmacol. 169, 1–10.

Dunn, R.W., Flanagan, D.M., Martin, L.L. et al., 1992. Stereoselective R-(+) enantiomer of HA-966 displays anxiolytic effects in rodents. Eur. J. Pharmacol. 214, 207.

Fraser, C.M., Cooke, M.J., Fisher, A. et al., 1996. Interactions between ifenprodil and dizolcipne on mouse behaviour in models of anxiety and working memory. Eur. Neuropsychopharmacol. 6 (4), 311–316.

Graeff, F.G., 1993. Role of 5-HT in defensive behavior and anxiety. Rev. Neurosci. 4, 181–211.

Grotta, J., Clark, W., Coull, B. et al., 1995. Safety and tolerability of the glutamate antagonist CGS 19755 in patients with acute ischemic stroke. Results of a phase IIa randomized trial. Stroke 26, 602–605.

Haefely, W., 1990. The GABA-benzodiazepine interaction fifteen years later. Neurochem. Res. 15 (2), 169–174.

Helton, D.R., Tizzano, J.P., Monn, J.A. et al., 1997. Anxiolytic and Side-effect profile of LY354740: a potent highly selective orally active agonist for group II metabotropic glutamate receptors. J. Pharmacol. Exp. Ther. 284, 651–660.

Hollmann, M., Heinemann, S., 1994. Cloned glutamate receptors. Annu. Rev. Neurosci. 17, 31–108.

Javitt, D.C., Zukin, R.C., 1991. Recent advances in the phencyclidine model of schizophrenia. Am. J. Psychiatry 148 (10), 1301–1308.

Jessa, M., Nazar, M., Bidzinsky, A., Plaznik, A., 1996. The effect of repeated administration of diazepam, MK-801 and CGP 37849 on rat behaviour in two models of anxiety. Eur. Neuropsychopharmacol. 6, 55–61.

Johnston, G.A.R., 1996. GABAa receptor pharmacology. Pharmacol. Ther. 69 (3), 173–198.

Jones, B.J., Blackburn, T.P., 2002. The medical benefit of 5-HT research. Pharmacol. Biochem. Behav. 71 (4), 555–568.

Kaczmarek, L., Kossut, M., Skangielkramska, J., 1997. Glutamate receptors in cortical plasticity: molecular and cellular biology. Physiol. Rev. 77, 217–255.

Karcz, M., Kubich, M., Liljequist, S., 1995. Evidence for an anxiogenic

action of AMPA receptor antagonists in the plus-maze test. Eur. J. Pharmacol. 279, 171–177.
Karcz, M., Jessa, M., Nazar, M. et al., 1997. Anxiolytic activity of glycine B antagonists and partial agonists-no relation to intrinsic activity in patch clamp. Neuropharmacology 36 (10), 1355–1367.
Kehne, J.H., McCloskey, T.C., Baron, B.M., Chi, E.M. et al., 1991. NMDA receptor complex antagonists have potential anxiolytic effects as measured with separation-induced ultrasonic vocalisation. Eur. J. Pharmacol. 193, 283–292.
Kehne, J.H., Baron, B.M., Harrison, B.L. et al., 1995. MDL 100, 458 and MDL 102,288: two potent and selective glycine receptor antagonists with different functional profiles. Eur. J. Pharmacol. 284, 109–118.
Kim, M., McGaugh, J.L., 1992. Effects of intra-amygdala injections of NMDA receptor antagonists on acquisition and retention of inhibitory avoidance. Brain Res. 58, 35–48.
Klepstad, P., Maurset, A., Moberg, E.R. et al., 1990. Evidence of a role for NMDA receptors in pain perception. Eur. J. Pharmacol. 187, 513–518.
Klodzinska, A., Chojnacka-Wojcik, E., Palucha, A. et al., 1999. Potential anti-anxiety, anti-addictive effects of LY354740, a selective group II glutamate metabotropic receptors agonist in animal models. Neuropharmacology 38, 1831–1839.
Koek, W., Colpaert, F.C., 1991. Use of a conflict procedure in pigeons to characterize anxiolytic drug activity: evaluation of NMDA antagonists. Life Sci. 49, 37–42.
Kotlinska, J., Liljequist, S., 1998a. The putative AMPA receptor antagonist, LY326325, produces anxiolytic effects without altering locomotor activity in rats. Pharmacol. Biochem. Behav. 60, 119–124.
Kotlinska, J., Liljequist, S., 1998b. A characterization of anxiolytic like action induced by the novel NMDA/glycine site antagonist L-701,324. Psychopharmacology (Berlin) 135 (2), 175–181.
Krystal, J.H., Laurence, P.K., Seibyl, J.P. et al., 1994. Subanesthetic effect of the non-competitive NMDA antagonist, ketamine, in humans. Psychomimetic, perceptual, cognitive and neuroendocrine responses. Arch. Gen. Psychiatry 51, 199–214.
Krystal, J.H., D'Souza, D.C., Ismene, L. et al., 1999. NMDA agonists and antagonists as probes of glutamergic dysfunction and pharmacotherapies in neuropsychiatric disorders. Harv. Rev. Psychiatry 7 (3), 125–143.
LeDoux, J.E., 1994. Emotion, memory and the brain. Sci. Am. 4, 32–39.
LeDoux, J.E., 1998. Fear and the brain: where have we been and where are we going? Biol. Psychiatry 44, 1229–1238.
Luby, E.D., Cohen, B.D., Rosenbaum, G. et al., 1959. Study of a new schizophrenic-mimetic drug: Sernyl. AMA Arch. Neurol. Psychiatry 81, 363–369.
Maren, S., 1996. Synaptic transmission and plasticity in the amygdala. Mol. Neurobiol. 13, 1–22.
Miserandino, M.J.D., Sananes, C.B., Melia, K.R., Davis, M., 1990. Blocking of acquisition but not expression of conditioned fear-potentiated startle by NMDA antagonists in the amygdala. Nature 345, 716–718.
Nakanishi, S., 1992. Molecular diversity of glutamate receptors and implications for brain function. Science 258, 597–603.
Ninan, P.T., Insel, T.M., Cohen, R.M. et al., 1982. Benzodiazepine receptor-mediated experimental anxiety in primates. Science 218, 1332–1334.
Ninan, P.T., 1999. The functional anatomy, neurochemistry, and pharmacology of anxiety. J. Clin. Psychiatry 60 (22), 12–17.
Ohishi, H., Shigemoto, R., Nakanishi, S., Mizuno, N., 1993. Distribution of the mRNA for a metabotropic glutamate receptor, mGluR2, in the central nervous system of the rat. Neuroscience 53, 1009–1018.
Parsons, C.G., Danysz, W., Quack, G., 1999a. Glutamate in CNS disorder as a target for drug development: an update. Drug News Perspect. 11 (9), 523–569.
Parsons, C.G., Danysz, W., Quack, G., 1999b. Memantine is a clinically well tolerated *N*-methyl-D-aspartate (NMDA) receptor antagonist—a review of preclinical data. Neuropharmacology 38, 735–767.
Pilc, A., Klodzinska, A., Branski, P. et al., 2002. Multiple MPEP administrations evoke anxiolytic- and antidepressant-like effects in rats. Neuropharmacology 43 (2), 181–187.
Pin, J.P., Duvoisin, R., 1995. The metabotropic glutamate receptors: structure and functions. Neuropharmacology 34, 1–26.
Pin, J.P., De Colle, C., Bessis, A.S., Acher, F., 1999. New perspectives for the development of selective metabotropic glutamate receptor ligands. Eur. J. Pharmacol. 375 (1–3), 277–294.
Plaznik, A., Palejko, W., Nazar, M., Jessa, M., 1994. Effects of antagonists at the NMDA receptor complex in two models of anxiety. Eur. Neuropsychopharmacol. 4, 503–512.
Porter, J.H., Wiley, J.L., Balster, R.L., 1989. Effects of phencyclidine-like drugs on punished behaviour in rats. J. Pharmacol. Exp. Ther. 248, 997–1002.
Przegalinsky, E., Tatarczynska, E., Deren-Wesolek, A. et al., 1996. Anticonflict effect of a competitive NMDA receptor antagonist and a partial agonist at strychine-insensitive glycine receptors. Pharmacol. Biochem. Behav. 54 (1), 73–77.
Rogan, T.C., Staubli, U.V., LeDoux, J.E., 1997. Fear conditioning induces associative long-term potentiation in the amygdala. Nature 390, 604–607.
Silvestre, J.S., Nadal, R., Pallares, M., Ferre, N., 1997. Acute effects of ketamine in the holeboard, the elevated plus maze, and the social interaction test in Wistar rats. Depress. Anxiety 5, 29–33.
Salt, T.E., Eaton, S.A., 1995. Functions of ionotropic and metabotropic glutamate receptors in sensory transmission in the mammalian thalamus. Progr. Neurobiol. 48, 55–72.
Sajdyk, T.J., Shekhar, A., 1997. Excitatory aminoacid receptors in the basolateral amygdala regulate anxiety responses in the social interaction test. Brain Res. 764, 262–264.
Sanger, D.J., Jackson, A., 1989. Effects of phencyclidine and other *N*-methyl-D-aspartate antagonists on the schedule controlled behaviour. J. Pharmacol. Exp. Ther. 248, 1215–1221.
Schoepp, D.D., Conn, P.J., 1993. Metabotropic glutamate receptors in brain function and pathology. Trends Pharmacol. Sci. 14, 13–20.
Schoepp, D.D., Jane, D.E., Monn, J.A., 1999. Pharmacological agents acting at subtypes of metabotropic glutamate receptors. Neuropharmacology 38 (10), 1431–1476.
Shepard, J.D., Barron, K.W., Myers, D.A., 2000. Corticosterone delivery to the amygdala increases corticotropin-releasing factor mRNA in the central amygdaloid nucleus and anxiety-like behavior. Brain Res. 861 (2), 288–295.
Shekhar, A., Keim, S.R., 2000. LY354740, a potent group II metapbotropic glutamate receptor agonist prevents lactate-induced panic-like response in panic-prone rats. Neuropharmacology 39, 1139–1146.
Spooren, W.P., Vassout, A., Neijt, H.C. et al., 2000. Anxiolytic-like effects of the prototypical metabotropic glutamate receptor 5 antagonist 2-methyl-6-(phenylethynyl)pyridine in rodents. J. Pharmacol. Exp. Ther. 295, 1267–1275.
Stephens, D.N., Meldrun, B.S., Weidmann, R., et al., 1986. Does the excitatory amino acid receptor antagonist 2-APH exhibit anxiolytic activity? Psychopharmacology 90, 166–169.
Tatarczynska, E., Klodzinska, A., Chojnacka-Wojcik, E., et al., 2001. Potential anxiolytic- and antidepressant-like effects of MPEP, a potent, selective and systemically active mGlu5 receptor antagonist. Br. J. Pharmacol. 132, 1423–1430.
Trullas, R., Jackson, B., Skolnick, P., 1989. Anxiolytic properties pr 1-aminocyclopropanecarboxylic acid, a ligand at strychnine-insensitive glycine receptors. Pharmacol. Biochem. Behav. 34, 313–316.
Vry De, J., Benz, U., Schreiber, R., Traber, J., 1993. Shock-induced ultrasonic vocalisation in young adult rats: a model for testing putative anxiolytic drugs. Eur. J. Pharmacol. 249, 331–339.
Walker, D.L., Davis, M., 2002. The role of amygdala glutamate receptors in fear learning, fear-potentiated startle, and extinction. Pharmacol. Biochem. Behav. 71, 379–392.
Watkins, J., Collingridge, G., 1994. Phenylglycine derivates as antagonist of metabotropic glutamate receptors. Trends Pharmacol. Sci. 15, 333–342.

Wiley, J.L., 1997. Behavioral pharmacology of NMDA antagonists: implications for the study and pharmacotherapy of anxiety and schizophrenia. Exp. Clin. Psychopharmacol. 5 (4), 365–374.

Wiley, J.L., Cristello, A.F., Balster, R.L., 1995. Effects of site selective NMDA receptor antagonists in an elevated plus model of anxiety in mice. Eur. J. Pharmacol. 294, 101–107.

Willetts, J., Clissold, T.L., Hartmann, R.R. et al., 1994. Behavioral pharmacology of NPC 17742, a competitive NMDA antagonist. J. Pharmacol. Exp. Ther. 265, 1055–1062.

Xie, Z., Commissaris, L., 1992. Anxiolytic-like effects of the noncompetitive NMDA antagonist MK801. Pharmacol. Biochem. Behav. 43, 471–477.